In Vivo Magnetic Resonance Imaging of Catheter-Based Vascular Gene Transfer

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Abstract-**The purpose of this study was to develop an in vivo imaging tool to monitor vascular gene transfer. We produced gadolinium/blue-dye and gadolinium/gene-vector media by mixing Magnevist with a trypan-blue or a lentiviral vector carrying a green fluorescent protein (GFP) gene. The gadolinium was used as an imaging marker for MRI to visualize vessel wall enhancement, while the blue-dye/GFP was used as a tissue stain marker for histology/immunohistochemistry to confirm the success of the transfer. Using Remedy gene delivery catheters, we transferred the gadolinium/blue-dye (n=8) or gadolinium/GFP-lentivirus (n=4) into the arteries of 12 pigs, monitored under high-resolution MR imaging. This technical development enabled dynamic visualization of: (i) where the gadolinium/genes distributed; (ii) how satisfactorily the target portion was marked; and (iii) whether the gene transfer procedure caused complications. Our study represents the first direct evidence that catheter-based vascular gene delivery/distribution can be monitored by MR imaging in vivo.**
Key wards: cardiovascular disease, magnetic resonance imaging, vascular gene therapy

I. INTRODUCTION

Atherosclerotic cardiovascular disease remains the leading cause of mortality in the United States[1]. Gene therapy is a rapidly expanding field with great potential for the treatment of cardiovascular diseases. Many genes have been shown to be useful for preventing acute thrombosis, blocking post-angioplasty restenosis, and stimulating growth of new blood vessels (angiogenesis)[2, 3].

Different gene delivery techniques for the vasculature have been developed, including: 1) ex vivo gene delivery, such as endovascular stents seeded with genetically modified endothelial cells which are then reimplanted into the target vessel; 2) surgery-based delivery, which involves directly injecting genes into surgically-isolated target vessels; 3) percutaneous delivery, which involves the direct administration of genes into the target through a percutaneous approach; and 4) catheter-based delivery[4]. Of these techniques, the catheter-based approach seems to hold the most promise for vascular applications[5].

Catheter-based gene delivery has some prominent advantages over other gene delivery methods, including (a) precise gene delivery to a specific anatomic location, (b) minimal morbidity, (c) no unwanted systemic effects, and (d) the ability to combine with conventional interventions, such as angioplasty and stent placement. However, gene transfer with these delivery catheters are currently performed under x-ray fluoroscopy, which displays, using a contrast medium, only the lumen of the vessel without providing direct imaging information about the vessel wall or atherosclerotic plaques. Therefore, one cannot properly monitor either the interaction between the genes and the atherosclerotic lesion or the existence and distribution of the genes within the target lesion during and after vascular gene transfer.

Monitoring catheter-based vascular gene delivery/distribution is critical. After the vascular gene transfer, clinicians need to immediately assess the success of the primary gene therapy procedure, including confirmation of where the genes go and how the genes target the atherosclerotic lesions. Unrecognized failure of the primary vascular gene transfer can delay treatment for several months. However, proven failure of the primary gene transfer should indicate the need for a prompt decision to replace the unsuccessful gene therapy with alternative treatment. Currently, we are unable to assess the success of the primary gene transfer procedure because no in vivo imaging modalities are available for vascular gene therapy. Thus, we need an imaging technique with high resolution imaging capability to visualize the vessel wall and the interaction between genes and the target atherosclerotic plaques. Cardiovascular MR technology offers great potential to fill this gap.

Cardiovascular MRI technology has some prominent advantages: possible imaging of the vessel wall and multiple diagnostic evaluations of organ function and morphology as well as multiple image planes with no risk of ionizing radiation. To date, MR imaging has been primarily used for investigations of non-cardiovascular gene therapies[6, 7] and its use for vascular gene therapy has not been reported. Here, we present our recent technical development using the MR imaging technique as an in vivo imaging tool to monitor catheter-based vascular gene transfer.

II. METHODOLOGY

One of the primary goals of our laboratory is to apply medical imaging technologies to vascular gene therapy. The present study included two sections. First, to establish the experimental protocol, we produced a gadolinium/blue-dye medium by mixing Magnevist (a gadolinium-based MR imaging dye) with trypan-blue medium. Second, to preclinically validate the technical development, we produced a gadolinium/gene-vector medium by mixing Magnevist with a lentiviral vector carrying a green fluorescent protein (GFP) gene. In the media, the gadolinium was used as an imaging marker for MRI to visualize the enhancement of the target vessel wall, while the blue-dye/GFP was used as a tissue/biology stain marker for histology/immunohistochemistry to confirm the success of the gadolinium/blue-dye/GFP-lentivirus-mediated transfer. Using a Remedy gene delivery catheter (Fig. 1), we transferred the gadolinium/blue-dye (n=8) or gadolinium/GFP-lentivirus (n=4) into the vessel walls of the iliac or femoral arteries of pigs under high resolution MR imaging. The contralateral vessels of the target arteries were not transferred to serve as controls.

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25 Oct 2001	N/A	-

Title and Subtitle In Vivo Magnetic Resonance Imaging of Catheter-Based Vascular Gene Transfer	**Contract Number**
	Grant Number
	Program Element Number
Author(s)	**Project Number**
	Task Number
	Work Unit Number
Performing Organization Name(s) and Address(es) Department of Radiology Johns Hopkins Hospital Baltimore, MD	**Performing Organization Report Number**
Sponsoring/Monitoring Agency Name(s) and Address(es) US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	**Sponsor/Monitor's Acronym(s)**
	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom., The original document contains color images.

Abstract

Subject Terms

Report Classification unclassified	**Classification of this page** unclassified
Classification of Abstract unclassified	**Limitation of Abstract** UU

Number of Pages
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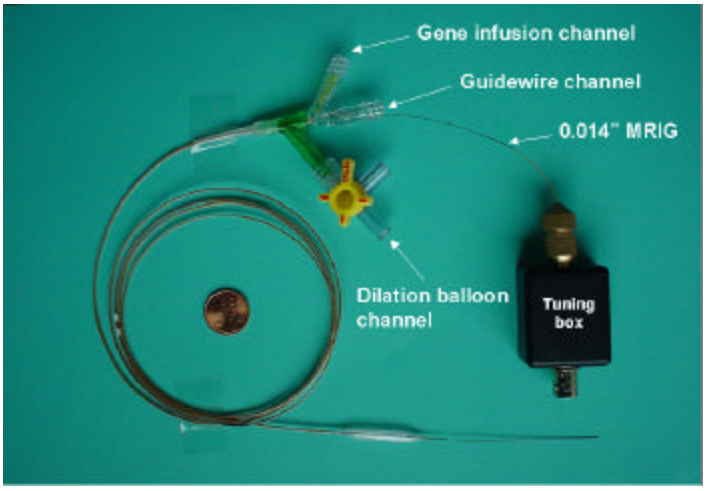

Fig. 1. A Remedy gene delivery balloon catheter with three channels: a dilation (angioplasty) balloon channel, a gene infusion channel, and a guidewire channel. An 0.014" MR imaging-guidewire (MRIG) is placed within the guidewire channel.

Gadolinium/gene-vector medium: We constructed a novel lentiviral vector (EF.GFP) containing the human elongation factor (EF)-1a promoter and the GFP gene (Y. Cui et al., manuscript submitted). Then, we mixed Magnevist with the GFP-lentiviral vector to achieve a gadolinium/GFP-lentivirus medium with a net Magnevist concentration of 6%. We have previously confirmed that 3-6% Magnevist (14-28 mg gadolinium/ml) is an optimum concentration for MR imaging of balloon inflation[8].

Gene delivery catheter positioning: We used 12 Remedy gene delivery balloon catheters, 3.4-4 mm in diameter (SCIMED, Maple Grove, Minnesota)(Fig. 1). This type of gene delivery catheter has the dual capability of high-pressure lesion dilation and low-pressure gene infusion. We first obtained a conventional X-ray angiogram to delineate the anatomy of the pelvic arterial tree of 12 anesthetized domestic pigs, 20-25 kg in weight. On the angiographic images, we selected a 3.0-mm (for a 3.5-mm balloon catheter) or 3.5-mm (for a 4-mm balloon catheter) vessel segment of either the iliac artery or the femoral artery on the left side. Then, the selected gene delivery catheter was positioned, via an 0.014-inch conventional guidewire, into the target arterial segment under x-ray fluoroscopy. Subsequently, we replaced the conventional guidewire with an 0.014-inch intravascular MR imaging-guidewire (Surgi-Vision, Columbia, Maryland) (Fig. 1).

MR imaging: All experiments were performed on a 1.5 Tesla MR unit (GE, Milwaukee, Wisconsin). To image deeply-located iliac arteries, we operated the MR imaging-guidewire at a receive-only mode, while to image the superficially-located femoral arteries, we used a custom-made, 3-cm surface coil. We first inflated the dilation balloon with 3% Magnevist and obtained a coronal scout MR image of the pelvis using a fast spoiled-gradient (FSPGR) pulse sequence, 500/2.1-msec repetition time (TR)/echo time (TE), 31.2-kHz bandwidth (BW), 24x24-cm field of view (FOV), 256x256 matrix, and 3 mm thickness. Based on this image, which demonstrated the gadolinium-inflated balloon, we then acquired an axial high-resolution MR image of the target arterial wall using: (i) a spin-echo (SE) sequence (with 150/10-msec TR/TE, 16-kHz BW, 6x6-cm FOV, 128x256 matrix, 1-3 number of excitation (NEX), and 3 mm thickness); and (ii) a FSPGR sequence (with 14.8/4.9-msec TR/TE, 15.6-kHz BW, 4x4 FOV, 256x256 matrix, 8 NEX, and 3 mm thickness). The total scan time for each image was 1 minute. During the infusion of the gadolinium/blue-dye/GFP-lentivirus medium, the dilation balloon was inflated at 4 atm support pressure, and the medium infusion was constantly maintained using a pump (Harvard, Holliston, Massachusetts) at different infusion flows of 5, 10, or 20 ml/hour and infusion times of 5, 10, or 15 minutes. During the infusion, we continuously acquired the axial high-resolution MR images at 3-minute intervals during the 0-15 minute infusion, and at 5-minute intervals for 15-30 minutes, as well as at 10-minute intervals for the 40-50 minutes after the infusion.

Histological and immunohistochemical confirmation: In the pigs infused with gadolinium/blue-dye, we immediately harvested the target vessels for histopathological examination to confirm the success of the transfer. The pigs infused with gadolinium/GFP-lentivirus were kept alive for five days to allow sufficient GFP expression. Then, we harvested the target arteries for immunohistochemical confirmation using a specific monoclonal antibody for GFP (Roche, Indianapolis, Indiana)[9].

Image analysis: On the axial high-resolution MR images, we visualized the gadolinium enhancement and distribution in the target vessel wall and its surrounding tissues. A region of interest was placed on the chosen portion of the target vessel wall to measure the MR signal intensities. Then, we converted the measurements to obtain a signal intensity versus time curve. The data was expressed as mean±standard deviation (SD).

III. RESULTS

First, in a series of pilot studies in the eight pigs, we tested and modified the experimental protocols for both gadolinium/blue-dye transfer and high-resolution MR imaging. In two pigs, we initially infused the gadolinium/blue medium at a 5-ml/hour flow for 5 minutes under MR imaging. With these infusion parameters, we detected only mild and partial enhancements of the target vessel walls, which was confirmed by histology as several blue-dye stain spots in the intima and its nearby media. We then increased the infusion flow to 10 ml/hour for 10-15 minutes in four pigs. At this time, we were able to dynamically visualize the gadolinium enhancement within the entire vessel wall (Fig. 2). The corresponding histology showed that the blue-dye located predominantly in the intima and media layers, presenting as the blue-stained cytoplasm and nuclei of endothelial cells and smooth muscle cells, as well as the internal elastic lamina (Fig. 2).

Subsequently, in the remaining two pigs, we tested the infusion of the gadolinium/blue medium at a higher flow of 20 ml/hour for 15 minutes. Under this condition, most of the gadolinium flowed into the areas outside the target vessels (Fig. 3), which, upon surgical examination, correlated with the blue-dye stain in the muscles adjacent to the target vessels (Fig. 3).

In addition, we tested different MR parameters. We found that high-resolution MR imaging using SE and FSPGR sequences were best for the purposes of this work. Thus, we used transfer parameters of 10-ml/hour flows for 10-15 minutes and high-resolution MR imaging with SE and FSPGR sequences in the subsequent in vivo validation studies using gadolinium/GFP-lentivirus. Since this study focused only on technical

development, we did not attempt to perform more quantitative experiments.

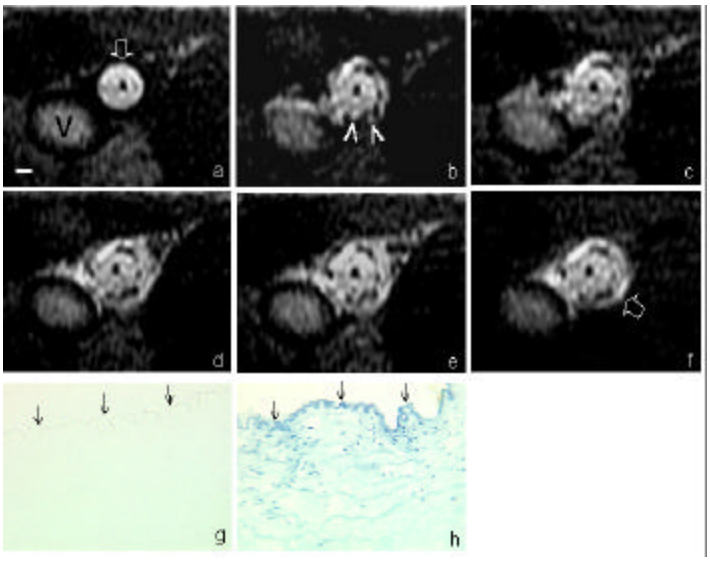

Fig. 2. High-resolution MR images of the gadolinium/trypan-blue transfer in the iliac artery of a pig. ***a,*** Before gadolinium/blue-dye infusion, the balloon is inflated with 3% Magnevist. The open arrow indicates the artery. V= the vein. Scale=1 mm. ***b-f,*** During gadolinium/blue-dye infusion from minute 3 to minute 15 (at three-minute intervals), the arterial wall is enhanced by the gadolinium coming from the gene infusion channels (arrowheads in ***b***) of the gene delivery catheter. At minute 15, the arterial wall is enhanced as a ring (arrow in ***f***). ***g*** and ***h,*** Corresponding histology of the target artery. ***h.*** The intima (arrows) and media are stained with blue color, which confirms the successful transfer of the gadolinium/trypan-blue medium. ***g,*** The artery without gadolinium/blue-dye transfer serves as a control. Original magnification, 200X.

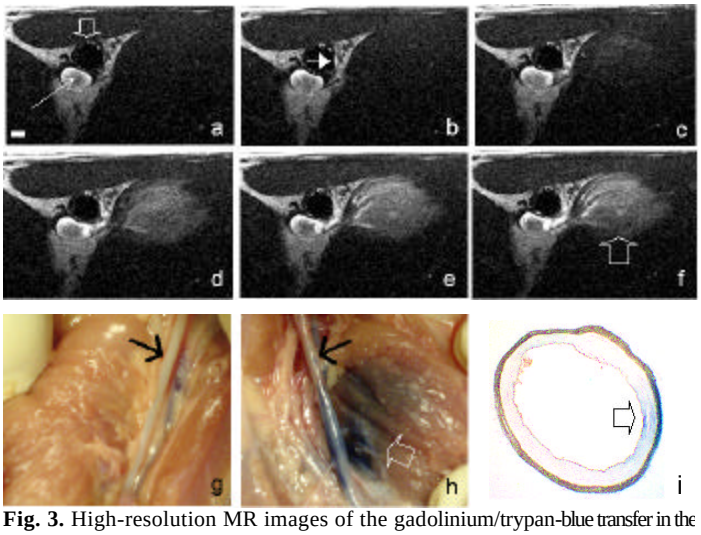

Fig. 3. High-resolution MR images of the gadolinium/trypan-blue transfer in the femoral artery of a pig. ***a,*** Before gadolinium/blue-dye infusion. The open arrow indicates the artery and the long arrow indicates the vein. Scale=1 mm. ***b-f,*** During gadolinium/blue-dye infusion from minute 3 to minute 15 (at three-minute intervals), the arterial wall is only partially enhanced by the gadolinium at 2-4 o'clock (arrow in ***b***). Gadolinium flow into the adjacent tissue (arrow in ***f***) outside the target artery. ***g-i,*** Corresponding surgery and histology of the target artery. ***h,*** The blue-dye stains the muscles (open arrow) outside the target artery (block arrow). There is no such finding in the control side (***g***). ***i,*** Histology confirms the blue-dye mainly stains the partial artery wall at 2-4 o'clock, with the wall dissection (arrow). Original magnification, 20X.

Next, we performed the preclinical validation studies using the above-described medium transfer/MR imaging protocol in four pigs. On MR imaging, the target vessel walls began to be enhanced immediately after beginning the gadolinium/GFP-lentivirus infusion (Fig. 4). The average time period for the target vessel walls to maintain peak signal intensity was from minute 6±2 (SD) to minute 20±3 (SD) after initiation of the gadolinium/GFP-lentivirus infusion (Fig. 5). After the gadolinium/gene transfer, all four pigs recovered. We did not detect any clinical signs of ischemia in the treated extremities during the five post-procedure days.

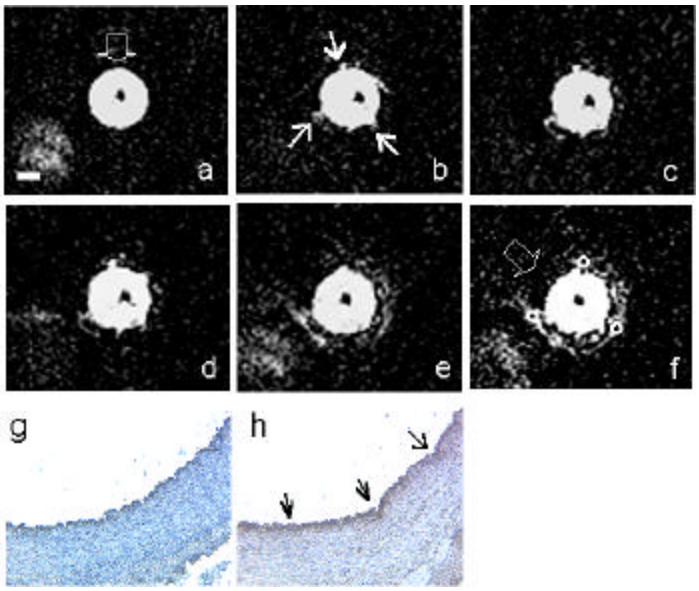

Fig. 4. High-resolution MR images of the gadolinium/GFP-lentivirus transfer in the iliac artery of a pig. ***a,*** Before gadolinium/GFP-lentivirus infusion. The arrow indicates the artery. Scale=1 mm. ***b-f,*** During gadolinium/GFP-lentivirus infusion from minute 3 to minute 15 (at three-minute intervals), the arterial wall is enhanced by the gadolinium coming from the gene infusion channels (arrows on ***b***) of the gene delivery catheter. After terminating the gadolinium/GFP-lentivirus infusion, part of the target arterial wall is not enhanced at 9-12 o'clock (arrow in ***f***). The three white circles in ***f*** present three regions of interest where MR signal intensity is measured. ***g*** and ***h,*** Corresponding immunohistochemical staining of GFP in the target artery. ***g,*** The untransferred artery as a control. ***h,*** The gadolinium/GFP-lentivirus medium has been delivered into the arterial wall. GFP is detected as brown-colored precipitates that result in a color change of the entire arterial wall from blue (***g***) to brown (***h***). GFPs locate predominantly in the intima (arrows). Original magnification, 200X.

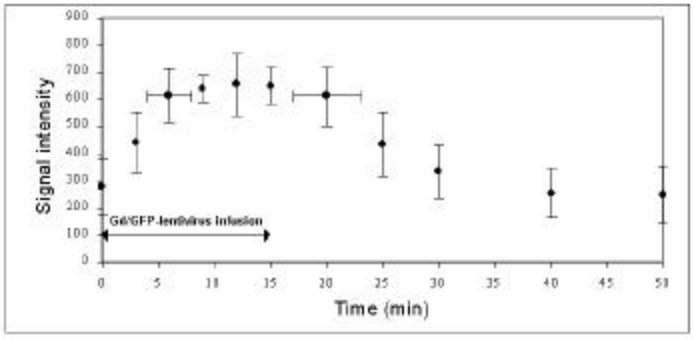

Fig. 5. A curve of MR signal intensity of the target vessel wall versus the infusion time of the gadolinium (Gd)/GFP-lentivirus transfer. The average time period for the target vessel walls to maintain peak signal intensity was from minute 6±2 (SD) to minute 20±3 (SD) after initiation of the gadolinium/GFP-lentivirus infusion, and then the signal intensity dropped to the pre-infusion level within 40-50 minutes.

The subsequent laboratory examination showed 100% correlation between the gadolinium enhancement of the target vessel walls on MR imaging and the GFP staining of the target vessel tissues with immunohistochemistry (Fig. 4). The expression of GFP determined in situ by immunohistochemistry indicated a functional in vivo gene transfer and expression with our approach. The immunohistochemistry showed that GFPs expressed predominantly in the intima and media, demonstrated as the color of the entire vessel specimens changed from blue (no GFP) to brown (due to GFP expression).

IV. DISCUSSION

Gene therapy is an exciting frontier in medicine today. Modern imaging techniques provide the opportunity to monitor and control gene therapy. Recent studies have shown that a gene therapy procedure can be demonstrated with imaging techniques, such as nuclear imaging[10, 11], optical imaging[9, 12], and MR imaging[6, 7]. However, to date, most investigations about imaging of gene therapy have primarily focused on non-cardiovascular systems, and no in vivo imaging modalities are currently available for monitoring of vascular gene therapy.

Our study represents the first direct evidence, to our knowledge, that catheter-based vascular gene delivery/distribution can be monitored by MR imaging in vivo. This technical development holds promise for in vivo assessment of the success of the primary vascular gene transfer procedure. This imaging method enables us to dynamically visualizes: (i) where the gadolinium/gene distributes, (ii) how satisfactorily the target portion of the vessel wall is marked, and (iii) whether the gene transfer procedure causes complications, such as micro-perforation of the target vessel wall. This should help clinicians to determine the reasons for a failed vascular gene transfer procedure, and promptly decide further management by either repeating the same gene therapeutic procedure or choosing an alternative treatment.

In addition, this technical development provides an in vivo imaging tool to prove the efficiency and reliability of the gene delivery catheters manufactured by different companies. An unexpected distribution of the gadolinium/gene-vector medium may indicate problems with the gene delivery catheter itself.

There have been several reports on vascular gene delivery using different catheters, including the Remedy gene delivery catheter, which has been tested at infusion times from 1 minute to 30 minute[13, 14]. However, standard gene delivery parameters, such as applied transmural pressure, viral solution volume and concentration, as well as gene delivery flow rate and infusion time, have not been established. The present technique should provide an imaging method that will enable selection of the best vascular gene transfer protocol and therefore regulate vascular gene therapeutic procedures.

V. CONCLUSION

We present a technical development using high-resolution MR imaging as an in vivo imaging tool to monitor catheter-based vascular gene transfer. We believe that this work opens up an exciting avenue for the future efficient management of cardiovascular ischemic disorders using MR imaging-based vascular gene therapy.

ACKNOWLEDGMENT

This study was supported in part by the Cardiovascular Interventional Radiology Research and Education Foundation; National Institutes of Health grants R01 HL67195, R01 HL61672, and P20 CA86346, as well as Johns Hopkins University Institutional Research Grants.

REFERENCES

[1] American Heart Association. "2001 heart and stroke statistical update," Dallas, TX.: American Heart Association.

[2] E. Nabel, "Gene therapy for cardiovascular disease," *Circulation*, vol. 91, pp. 541-548, 1995.

[3] J. Isner, "Manipulating angiogenesis against vascular disease," *Hosp Pract*, vol. 34, pp. 69-74, 1999.

[4] J. Thomas, M. Kuo, M. Chawla, J. Waugh, E. Yuksel, K. Wright, P. Gerrity, S. Shenaq, C. Whigham, and R. Fisher, "Vascular gene therapy," *RadioGraphics*, vol. 18, pp. 1373-1394, 1998.

[5] K. Kandarpa, "Catheter-based techniques for endovascular local drug delivery," *JVIR (supplement)*, vol. 11, pp. 419-423., 2000.

[6] G. Johnason, H. Benveniste, R. Black, L. Hedlund, R. Maronpot, and B. Smith, "Histology by magnetic resonance microscopy," *Magn Rson Q*, vol. 9, pp. 1-30, 1993.

[7] R. Weissleder, A. Moore, U. Mahmood, R. Bhorade, H. Benveniste, A. E. Chiocca, and J. P. Basilion, "In vivo magnetic resonance imaging of transgene expression," *Nature Medicine*, vol. 6, pp. 351-354, 2000.

[8] X. Yang and E. Atalar, "Intravascular MR-guided balloon angioplasty using an MR imaging-guidewire: An in vivo feasibility study," *Radiology*, vol. 217, pp. 501-506, 2000.

[9] X. Yang, H. Liu, D. Li, Z. Zhou, W. Jung, A. Deans, Y. Cui, and L. Cheng, "Digital optical imaging of green fluorescent proteins for tracking vascular gene expression: Feasibility study in rabbit and human cell models," *Radiology*, vol. 219, pp. 171-175, 2001.

[10] J. Tjuvajev, G. Stochhammer, R. Dsai, H. Uehara, K. Watanabe, B. Gansbacher, and R. Blasberg, "Imaging the expression of transfected genes in vivo.," *Cancer Res*, vol. 55, pp. 6126-6132., 1995.

[11] Y. Yu, A. J. Annala, J. R. Barrio, T. Torokuni, N. Satyamurthy, M. Namavari, S. R. Cherry, M. E. Phelps, H. R. Herschman, and S. S. Gambhir, "Quantification of target gene expression by imaging reporter gene expression in living animals," *Nature Medicine*, vol. 6, pp. 933-937, 2000.

[12] P. Contag, I. Olomu, D. Stevenson, and C. Contag, "Bioluminescent indicators in living mammals.," *Nature Medicine*, vol. 4, pp. 245-247., 1998.

[13] L. Maillard, E. Van-Belle, F. Tio, A. Rivard, M. Kearney, D. Branellec, P. Steg, J. Isner, and K. Walsh, "Effect of percutaneous adenovirus-mediated Gas gene delivery to the arterial wall in double-injured atheromatous stented rabbit iliac arteries," *Gene Therapy*, vol. 7, pp. 1353-1361, 2000.

[14] O. Varenne, R. Gerard, P. Sinnaeve, H. Gillijns, D. Collen, and S. Janssens, "Percutaneous adenoviral gene transfer into porcine coronary arteries: Is catheter-based gene delivery adapted to coronary circulation?," *Human Gene Therapy*, vol. 10, pp. 1105-1115, 1999.